ON THE ETIOLOGY OF TYPHUS FEVER.*

By Edwin W. Schultz, M.D.,
STANFORD UNIVERSITY, CALIFORNIA.

For more than fifty years scientists have sought for the etiologic agent in typhus fever and still the cause remains undiscovered. During the past thirty years there have been more than twenty organisms held forth by various workers as the cause, or probable cause, of typhus fever. It is not my purpose to review the enormous literature which has accumulated from the earlier studies, interesting as that might prove to be; however, in view of the fact that the Great War somewhat interfered with the American profession keeping abreast with the contributions made along these lines in Europe, especially in Germany, it seems worth while to present the more important contributions which grew out of the serious typhus situation in Eastern Europe and, what seems to be, the real status of our knowledge at the present time regarding the causative agent of this disease.

To some the discovery made by Plotz[24] in 1914, and subsequently enlarged by him and his associates, [1 23 25 26 27] seemed to be the final solution of this difficult problem. Indeed, there are authors of text-books in bacteriology who have practically accepted this organism as the cause of typhus fever. True, the organism seemed to have more in its favor than did even the most promising of those previously upheld as the cause of typhus fever, but it cannot be said that sufficient evidence has been adduced to justify its being named "B. typhi-exanthematici."

The organism which Plotz described is a small pleomorphic, Gram-positive bacillus, which is non-motile, not encapsulated and not acid-fast. It is an obligate anaërobe and requires a rather select culture medium. Its length is said to vary from 0.9 to 1.93 microns, its breadth from one-fifth to three-fifths its length. The organisms are straight; occasional ones are slightly curved. The ends are rounded or slightly pointed. Coccoid forms are said to occur. With special stains an occasional organism shows a small polar body at one end; more rarely at both ends. The colonies usually appear in the culture tubes in about ten days; occasionally as early as the fifth day or as late as the nineteenth day. They appear first as opaque spots, which by direct light appear white. Subsequently they assume a "Y"-shaped growth, surrounded with a zone of brownish precipitate. The organism has been isolated from both Brill's disease, the mild American form of typhus fever, and epidemic

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typhus fever. It has been grown from typhus infected animals and from infected lice. The organism shortly after isolation is slightly pathogenic for guinea-pigs; it loses its pathogenicity very rapidly. In the human host it induces the formation of specific agglutinins, precipitins, opsonins and complement-fixing antibodies. These are, however, detectable chiefly during the convalescent period. The agglutination readings average in the neighborhood of 1 to 200; only in one instance recorded going as high as 1 to 1600. These shall be discussed more fully further on.

In spite of the elaborate studies made by Plotz and his associates, their results cannot be said to show more than the fact that the organism is found in typhus fever patients. This much we must acknowledge in face of the cultural results and the serologic studies. But simply because an organism is found in a given disease does not license one to claim for it an etiologic role in the disease. The fact that it possesses a degree of pathogenicity for animals susceptible to the disease in question is of little significance unless the typical disease picture is reproduced. This includes not only the typical course of the disease but also the reaction induced by the disease, in other words, immunity relationships, if any occur. It is a well-known fact that typhus fever leaves an absolute immunity to further attacks in both man and animals. This immunity can be easily proved in animals by reinoculating them with the blood of a typhus fever patient subsequent to the animal's recovery from the disease, and in studying an organism isolated in typhus fever this procedure should be carried out extensively to determine whether or not it, in addition to producing the characteristic disease in a typhus susceptible animal (preferably a guinea-pig), also conveys definite immunity against subsequent inoculation with virulent typhus blood. Plotz and his associates did not succeed in reproducing even the disease picture in animals. The reason for this is laid to the extremely rapid loss of virulence of their organism on artificial media. But the fact remains that neither the disease picture nor what is more important, immunity reaction, was produced by inoculating their organisms into experimental animals. Their claim that the organism is the cause of the disease then rests solely upon cultural and serologic grounds and is unsupported by conclusive immunologic results.

In the autumn of 1915, Weil and Felix[46] isolated from the urine of typhus fever patients two strains of Proteus vulgaris which they named X1 and X2. These they found were markedly agglutinated by the sera from typhus fever patients, and on making control examinations learned that they were not dealing with ordinary saprophytes. The application of this discovery to the laboratory diagnosis of typhus fever was soon recognized by them. In the spring of 1916 they isolated another strain of this bacillus, also from the urine of a typhus fever patient, which they named X19.

This strain differed from the other two in that sera from typhus fever patients agglutinated the organism in dilutions many times higher than either of the other two—at that time one instance as high as 1 to 50,000. This organism has therefore, superseded the other two in the new serodiagnosis of typhus fever. It is not within the scope of this paper to discuss the technic of this method. In general it is not unlike the Gruber-Widal reaction. The reliability and diagnostic facility of this reaction has been confirmed by many workers and it has found a place in the diagnosis of typhus fever throughout Europe. It is diagnostic in practically 100 per cent. of the cases. The organism (Proteus X19) can be grown easily on ordinary nutrient media and may be kept for months without transplanting. In 1916 Dienes,[3 4] succeeded in isolating these X-strains from the blood of the typhus fever patients in 30 per cent. of cases which he examined. The reason why Proteus X19 has not been isolated in a higher percentage of cases was found by Felix[7] in 1917 to be due to the sensitiveness of the X-strain to the acid production of other bacteria in the urine and stools; to its sensitiveness to changes in media and, in the blood stream, to the rapid setting in of the bactericidal substances. Friedberger,[8] in 1917, on the basis of pathogenicity for guinea-pigs, agglutinins, precipitins, complement-fixation and Pfeiffer's phenomenon, maintained that Proteus X19 was probably the etiological agent in typhus fever. But in 1918 Landsteiner and Hausmann,[14] as well as Doerr and Pick,[5] and in 1919 Mollers and Wolff,[16] proved that Proteus X19 would not immunize guinea-pigs against typhus fever; that is, animals inoculated with blood from typhus fever patients subsequent to their recovery from Proteus X19 infection, developed typhus fever. We have here then an organism which, though occurring with remarkable regularity in typhus fever, apparently bears no etiologic relationship to the disease. This is especially interesting in view of the very pronounced serologic responses which the organism induces and the promptness with which the antibodies put in their appearance, generally within the first week of illness.

Now, let us compare the serologic responses induced by the Plotz bacillus with those produced by Proteus X19. It was previously stated that Plotz and his associates were unable to supply proof on immunologic grounds that their organism really was the cause of typhus fever and that the arguments for naming the organism "B. typhi-exanthematici" rest chiefly on a cultural and serologic foundation. The inadequacy of these arguments become obvious in a comparison of the agglutination readings of the two organisms in question. The average readings with the Plotz organism are figured from the readings given in one of Plotz's publications[25] and the average readings with Proteus X19 are obtained from one hundred readings made by Professor Oettinger[22] in studying the Weil-Felix reaction and the biology of Proteus X19. My figures show that

during the febrile period the Plotz organism is agglutinated by the patient's sera in dilutions of 1 to 8, at the time of crisis 1 to 113 and fifteen days after the crisis 1 to 220. The readings during the febrile period were for the most part negatives (readings below 1 to 50 were counted negatives), the highest reading being only 1 to 100. At the time of crisis they ran about half negatives, the highest reading being 1 to 800, and after the crisis they ran from negatives to 1 to 1400. Let us compare with these figures the averages of one hundred readings made by Oettinger in his studies on the Weil-Felix reaction. It will be observed that the agglutinins for X19 are far more concentrated and appear earlier in the disease. On the average eighth day of illness the agglutination readings average 1 to 2424, varying from 1 to 25 to 1 to 60,000 during the febrile period. On the average tenth day after the crisis the readings average 1 to 2540, one instance as high as 1 to 25,000 on the eleventh day. My own observation with the Weil-Felix reaction made while I was serving as bacteriologist for the American Red Cross Commission to Germany, satisfy me that the readings run a very high average, and in occasional cases exceptionally high. Indeed, a few of the workers have placed 1 to 200 as the lowest positive reading, which is practically as high as the average readings obtained with the Plotz organism at the time of crisis. It should be noted that the Plotz organisms show a degree of spontaneous agglutination which should also be allowed for in making this comparison. In short, we see that the agglutination readings obtained in using the Plotz bacillus are *nil* compared with those obtained in using Proteus X19, an organism which for these very reasons Freidberger felt justified in championing as the cause of the disease. Without proof on immunologic grounds and with serologic values which are negligible, what argument is there really left for upholding the Plotz bacillus as the cause of the disease?

Significance has also been attached to vague results supposed to have been obtained by attempted prophylactic immunization of people against typhus fever with the Plotz organism. In the winter of 1915–1916, Plotz, Olitsky and Baehr[26] inoculated 8420 persons, members of hospital, sanitation and other units in Serbia, Bulgaria and Volhynia, with a vaccine prepared from their organism. Out of this number six developed typhus fever during the four months of the epidemic. In other words the immunity, if any was really conveyed, was, at any rate, not absolute or complete. It therefore does not yield an added argument in favor of the organism being the cause of the disease. They were unable to obtain statistics as to the prevalence of the disease in the regions where they vaccinated so that one is at loss to even speculate on what possible decrease in the incidence of the disease might have resulted from these vaccinations. But it would seem that six cases among 8420 persons in a period of four months is not a negligible incidence of the disease.

Another important factor with which we are unfamiliar is the degree to which the various organizations enforced other prophylactic measures and the employment of typhus immune persons for the more hazardous duties in the handling and care of typhus fever patients, such as those of cleaning up the patients in the admitting office, etc. In short the results of the above experiments do not seem to furnish any added argument in favor of the Plotz organism being the etiologic agent.

But the question may be asked: Is not the fact that the Plotz organism has been grown from infected lice an excellent argument in favor of its being the causal agent of typhus fever? To this the reply would seem to be a question: Whence comes Proteus X19? It seems to me not improbable that the human infection is derived from the louse, and it is not unlikely that a number of other secondary organisms are introduced by the louse. The diplobacillus of Rabinowitsch,[30] also markedly agglutinated by the patient's sera, is probably another member of such a group. In brief, there are excellent reasons for believing that in typhus fever we have as a regular thing a state of mixed infection and that the organisms associated with the active virus of the disease are most probably introduced by the louse. There may be a state of symbiosis. It is worthy of note, in this connection, that Widmann,[49] in 1915 cultured a small Gram-negative, anaërobic, bacillus from normal lice. It is possible that quite an array of bacteria might be cultivated from both normal and infected lice.

This brings us to one of the most fascinating chapters in the history of research on the etiology of typhus fever, namely, that dealing with the development of our knowledge of the so-called Rickettsia. These studies were begun by Ricketts and Wilder[32] about ten years ago, but not until recently was there an appreciable advance made in our knowledge concerning them. Ricketts and Wilder,[32] in 1910, while working on the etiology of typhus fever in Mexico, made a large number of smear preparations from the digestive tracts of infected lice which they stained by the Giemsa method. They found that these smears contained large numbers of characteristic bipolar staining organisms. The thing that disturbed the significance of their observations at the time was the occasional occurrence of the same sort of organisms in smears which they prepared from normal lice. The fact that they dealt with two closely related organisms, one found exclusively in infected lice, has come to our notice through more recent investigations to be discussed presently. The observations of Ricketts and Wilder were confirmed by Prowazek[28] in 1913; by Sergent, Foley and Vialatte[39] in 1914, and by others.

In 1915 da Rocha-Lima[33 34 35] prompted by the earlier observations made on smear preparations of infected and normal lice, determined to study the lice by histologic methods, hoping that

this might clear up the confusion regarding these bodies. This proved to be a fruitful idea. It revealed the fact that a state of intracellular parasitism existed in typhus infected lice, involving the epithelial cells of particularly the gastrium. The control examinations showed that such a condition never occurred in non-infected lice—lice obtained from a typhus-free region. The involved cells of the gastric mucosa are found to undergo striking alterations so that their identification is frequently possible under the low power of a microscope. Within the cells the parasites, when few in numbers, are found to lie together in a group sharply rounded off from the rest of the cytoplasm. This is the picture early after an invasion, but as time goes on and the parasite multiplies the cells become entirely filled with the parasitic bodies and eventually cause them to be ballooned-out into the lumen of the stomach, protruding from the mucosa like droplets of fluid. Miss Sikora[40] observed the same involvement in the cells of the salivary glands. The parasite itself, da Rocha-Lima informs us, impresses one as a very small bacterium—smaller than M. melitensis or M. prodigiosus, but under very high magnification it appears not round but ellipsoid or olive-formed. These ellipsoid bodies lie for the most part in pairs, end to end, connected by a less intensely stained intermediate and surrounding substance. These double forms have the appearance of dumb-bells. Single forms also occur; the products of a recent division, but these are in the minority. Da Rocha-Lima estimates the single as 0.3 x 0.4 of a micron in size and the double or mature forms as 0.3 x 0.9 of a micron. Their staining qualities are of special interest. They stain very feebly or not at all with the ordinary bacterial stains, such as carbol-fuchsin, carbol-thionin, methylene blue, etc. They stain best and most characteristically with Giemsa, taking this stain differently than do bacteria. With this method they stain a pale ruby-red, almost the same coloration as nuclei take with this stain; however, not so bright a red, but more like the coloration which one obtains by this method on spirochetes and the tails of spermatozoa. Bacteria with this method generally stain a dark-red or an outspoken blue. The organism cannot be stained by Gram's method. Though many special methods were employed, all endeavors to cultivate the organism have failed. Da Rocha-Lima also noted the occasional occurrence of similar bodies in normal lice, but observed that these, without exception, lie extracellularly in the lumen of the digestive tract. These, undoubtedly, were the source of confusion in Ricketts' time when lice were studied entirely by smear preparations. Those of interest in connection with typhus fever are the intracellular forms found exclusively in typhus infected lice. For these da Rocha-Lima proposed the name, "Rickettsia-Prowazeki," in honor of these two scientists who gave their lives in studying the etiology of typhus fever. Other—what seem to be—species of this new group of

organisms have since been described, and to these I shall refer at the close of this review on the Rickettsia.

Let us now take up the evolution of this phenomenon of intracellular parasitism in infected lice. Da Rocha-Lima showed experimentally that the louse is incapable of transmitting the virus of typhus fever until at least five days after the louse has fed on a typhus fever patient. In other words a definite period for development or evolution is required by the organism in the intermediate host. Now, what light does da Rocha-Lima's histologic studies throw upon this phenomenon? Namely this, that the cells do not show an involvement previous to the fourth day after the louse has been infected, while after that period of time the phenomenon is present with regularity. His method of studying the development of the virus in lice is deserving of mention. The experimental lice were kept in a cleverly designed cage of very finely meshed screening (several of these which were left by him in Warsaw, Poland, at the time the Germans were driven from that place, were later utilized in our own studies). The cages containing the lice were tied to a patient twice a day for one hour and the rest of the time were kept in a thermostat at 33° C. He found that lice kept at a temperature of 23° C. between the usual feedings did not develop the Rickettsia and, furthermore, that injections of emulsions of the viscera of such lice into guinea-pigs did not result in an infection. Da Rocha-Lima showed also that the infection of the female louse may be inherited by its offspring.

Da Rocha-Lima[33] was the first to successfully infect guinea-pigs with typhus fever by direct inoculation of the virus in the form of emulsions prepared from the organs of virulent lice. The animals thus inoculated, after having undergone the usual course of the disease, were found to be immune to subsequent inoculation with blood taken from typhus fever patients. He found that the infectivity of such emulsions from lice was in direct proportion to the numerical content of Rickettsia. In one instance da Rocha-Lima succeeded in carrying on the louse virus, in passage from guinea-pig to guinea-pig, for nearly one year, during this time passing it through twenty-three animals. Blood from the eleventh passage was injected into a monkey, Macaccus sinicus, which developed the typical disease after an incubation period of eight days.

More interesting still are da Rocha-Lima's[33] studies on prophylactic immunization, using guinea-pigs as his subjects and a vaccine prepared from virulent lice. The lice used for the preparation of the vaccine were fed on typhus fever patients and kept under conditions which would ensure the greatest development of the Rickettsia. Emulsions were then prepared from the viscerae of these lice and the virus was attenuated by aging it for a number of weeks. The vaccine so prepared was administered hypodermically. The best results, absolute immunity of the entire series of animals, were

obtained by making three inoculations within the space of one week with a vaccine allowed to age for three weeks; the first dose containing an emulsion of five lice, the second ten and the third twenty lice. The experiments were controlled with normal guinea-pigs; the entire group, vaccinated and controls, received the usual infecting dose of blood from typhus fever patients. The non-vaccinated pigs contracted the disease, while the vaccinated ones, with a negligible exception, failed to show any reaction whatever.

It has been stated that other—what seem to be—species of Rickettsia occur in Nature. Before taking these up, let us review the features which separate the Rickettsia prowazeki from the rest, biologically, if not otherwise: (1) The Rickettsia prowazeki have a specific intermediate host, namely, Pediculus vestimenti. That the human louse is essential for the development of the Rickettsia-prowazeki was demonstrated by the researches of Nöller.[19] He fed pig lice and human lice on typhus fever patients at the same time. The Rickettsia developed in the human but not in the pig lice. There is one slight exception to this specificity, for Toepfer[42 43] and Toepfer and Schussler[44] whose studies confirmed for the most part da Rocha-Lima's work, claim to have observed the development of the Rickettsia prowazeki also in the human head louse, P. capitis. The biologic difference between P. vestimenti and P. capitis must be so small, if any, that the development of the Rickettsia prowazeki in the head louse need not be looked upon as a deviation from the specificity of the intermediate host. (2) The Rickettsia prowazeki are intracellular parasites of the louse.

Now let us turn briefly to the organism which was the source of confusion to Ricketts and Wilder and others since then, namely, the one occasionally found in normal lice. Nicolle, Blanc and Conseil,[17] working in Tunis in 1914, found that 5 per cent. of lice examined (smear preparations) in a typhus free region contained Rickettsia-like bodies. Brumpt,[2] in 1918, found them in smear preparations in 73 per cent. of lice taken from seven healthy war prisoners. This matter was also studied by da Rocha-Lima histologically. He found that whenever these bodies were found to occur in normal lice they were distributed over the surface of the gastric mucosa and never were found to lie within the cells even though they are found to lie in a rather thick layer against the mucosa. He further observed that though this organism was very similar to the Rickettsia prowazeki it was thicker and plumper and stained with greater ease than the Rickettsia prowazeki, though still not so easily as did the ordinary bacteria. Da Rocha-Lima named these Rickettsia-like organisms in normal lice, Rickettsia pediculi.

The same kind of organisms have also been found to occur in lice taken off patients suffering from Wolhynian fever, a disease similar to or synonymous with trench fever. These have been studied

particularly by Toepfer,[41] Jungmann[10 12] and by da Rocha-Lima.[15] They have been observed in as high as 80 per cent. of lice removed from patients at delousing stations, and are found to also occur in the form of a layer upon the gastric mucosa, though here one occasionally does observe a slight degree of intracellular invasion. Jungmann has named this organism, Rickettsia wolhynica.

None of the three above-named Rickettsia have yielded to artificial cultivation. This might cast a possible doubt on their actually being organisms, were it not for the fact that one species of this new group of organisms has recently been cultivated. The discovery was quite accidental. Nöller,[20 21] in 1917, while cultivating the Crithidia melophagi, a flagellate occurring in the sheep louse, Melphagus ovinus, found growing together with the Crithidia in separate colonies, a microörganism which in every respect looked like the Rickettsia. He named the organism Rickettsia melophagi. The following year Jungmann[13] confirmed and elaborated the studies of Nöller. The organism grows easily on the surface of a "sheep-serum-grape-sugar-agar" at 28° C. The colonies appear in six to eight days as minute, punctiform, translucent, round colonies, which on microscopic examination prove to be pure cultures of Rickettsia. Jungmann also studied the sheep lice histologically and found these bodies overlying the gastric mucosa, rarely noting even a slight intracellular involvement. He found that the infection was a constant one and, in a cleverly conducted research, established the fact that the lice obtained the infection by hereditary transmission and not from the blood of the sheep upon which they fed. The organism possesses absolutely no pathogenicity for any animals.

From the review of these researches on the Rickettsia we gather that an entirely new group of organisms has been discovered, a group seemingly occupying a position closer to protozoa than to bacteria.

It is thought by some that the Rickettsia prowazeki and the Plotz organism probably are one and the same organism. It is rather difficult to see wherein the likeness lies, and if the original description given by Plotz had not been later qualified in a remarkable way, there would, of course, have been no dispute as to their dissimilarity. Shortly after da Rocha-Lima's first publication on the Rickettsia appeared, Olitsky, Denzer and Husk[23] published the results of their studies in Mexico, in which they reported that they had grown the Plotz organism from infected lice. In this paper we get, for the first time, the remarkable fact that the Plotz organism is not always Gram-positive. They state: "In some of our blood cultures, however, smears made directly from colonies showed only bacilli which were completely decolorized by Gram's method. In other cultures the predominating organisms were Gram-positive, with numerous Gram-negative bacilli scattered throughout the

field. In subsequent subcultures the bacilli always became Gram-positive." Then they refer to the publication by da Rocha-Lima[33] as confirming their results. They state, referring to their cultures from lice, "In recent studies by da Rocha-Lima, further corroboration of this fact is made; he finds that infective lice harbor an organism of similar morphology in enormous numbers, especially in the stomach walls, and, remarkable to state, these organisms are Gram-negative." Strange to state, da Rocha-Lima does not acknowledge any such similarity. In the first place the Rickettsia are, strictly speaking, neither Gram-positive nor Gram-negative; they simply will not stain by Gram's method, because, for one thing, they do not stain readily with the simple bacterial stains. Outside of the unusual phenomenon with reference to the Gram-stain, Plotz does not mention any special staining qualities for his organism, and it is to be implied that it has the qualities of ordinary bacteria in respect to the simple stains. Besides, there is little in common morphologically between the two organisms. All attempts to grow the Rickettsia prowazeki seem to have failed in spite of the suggestions which might have grown out of the methods employed by Plotz and his associates. Finally, the one species of Rickettsia, Rickettsia melophagi, which has been cultivated, proved to be an aërobe, and it is not improbable, therefore, that the Rickettsia prowazeki is also an aërobe, rather than an anaërobe, as is the Plotz bacillus. The staining qualities, the sort of development in the louse, and the hereditary transmission of infection in lice resemble the activities of protozoa more closely than those of bacteria.

So much for the Rickettsia as observed in typhus infected lice. Though the grounds are less secure, it is, nevertheless, interesting to note the instances where Rickettsia-like organisms have been observed in the blood and tissues of typhus fever patients and experimental animals. Ricketts and Wilder[32] observed them in Giemsa-stained blood smears of typhus fever patients and in fresh preparations. They described the organism as a short bacillus which at first sight appeared solidly stained, but on minute examination showed an unstained or faintly stained zone across the middle. The fresh preparations also showed a differentiation of the forms into two halves, separated by a line or narrow zone of substance of a different refractive character, the organism possessing, not motility, but more or less rapid vibration. In 1911 Nicolle observed such bodies in the neutrophilic leukocytes of typhus infected chimpanzees, and Consiel and Conor[18] and Givino and Girard[9] in the same year in the neutrophilic leukocytes of typhus fever patients (Reder[31]). In 1913 Prowazek[28] made the same observations. He states that they occurred as oval and diplo-forms, lying in vacuoles, generally at the periphery of the leukocytes, and, with the Giemsa stain, colored a carmine-red, and were distinguishable from the neutrophilic granulations of the leukocyte. Da Rocha-Lima[36] has also observed

in blood smears from typhus fever patients as well as in histologic sections and smears made from tissues of typhus cadavers, structures which, in size, form and coloration, appeared the same as the Rickettsia. He tried out a large number of different staining methods, but was only able to demonstrate them when using the Romanowsky-Giemsa method.

In close association with the observation just described are those of Dr. Ludwig Anigstein, of the Central Epidemiologic Institute, at Warsaw, Poland, and myself, working together under the American Red Cross Commission to Poland. It was planned, as a part of our work, to carry on extensive studies on the distribution of these Rickettsia-like organisms in the tissues of typhus guinea-pigs and monkeys, and, as far as possible, human cadavers. But this part of the work did not materialize for reasons beyond our control. We were forced to limit our studies to blood smears and fresh blood from typhus fever patients. Doctor Anigstein prepared something like one hundred and fifty blood smears, representing about fifty distinct samples of blood from different periods of the disease. These he stained by various methods, chiefly with Giemsa and Leischmann stains. While these preparations were very interesting from the standpoint of striking nuclear changes, which will be written about later, only rarely were there bodies observed in the leukocytes which answered to Prowazek's description. One frequently sees, what seem to be exceptional inclusions of minute bodies, but to determine their morphology with accuracy is generally out of the question. However, bodies corresponding to the Rickettsia in shape, size and coloration were occasionally found lying free in the blood plasm.

To us the most interesting observations were those that we made with a dark-field, using a special Zeiss lens giving a magnification of at least 2000×. We examined by this method over fifty specimens of blood obtained from nearly as many different typhus fever patients in different stages of the febrile period of the disease. Of these 90 per cent. showed dancing bodies which in morphology, refractility and size were the same as those we observed in dark-field preparations of infected lice, which were made for comparison and afterward stained to further establish the presence of Rickettsia. These bodies occurred as single or ellipsoid forms but chiefly as the double or dumb-bell forms. It is extremely difficult to identify the single forms but the double forms are quite characteristic. The double forms really have the appearance of dumb-bells, two oval bodies being held together in their long axes by a less refractile fluid-like substance lying intermediately and frequently seen to surround the oval bodies. They are readily distinguished from the microsomes, or so-called blood-dust, in that these are much more refractile, generally larger and differ somewhat in morphology. As to numbers it was found that on

examining a capillary film between the slide and cover-slip under the magnification described, their number varied anything from one to five per field. No studies to determine their relative incidence in various periods of the disease were undertaken. The specimens were obtained by vena- and finger-puncture and special care was taken in the preparation of the glassware used. For financial reasons the work of the American Red Cross had to be materially curtailed and our work came to a premature close so that more extensive studies were out of the question. Twenty-five specimens of normal blood examined by the same method and with equal care failed to show these Rickettsia-like bodies.

I should like to now present certain arguments in favor of the etiologic agent of typhus fever being a protozoön or an organism related to the protozoa rather than a bacterium. In the first place the studies by da Rocha-Lima and others show that the louse as an intermediate host is not very unlike the anopheles mosquito in the spread of malaria. The louse does not serve as an intermediate host in the same way that the flea does in transmitting the Pest bacillus, by carrying the organism in its digestive tract. The phenomenon of intracellular parasitism in typhus infected lice, involving even the cells of the salivary glands, preceded by a definite period of development during which time the louse is non-infective, are features that one immediately associates with protozoan organisms. To this may be added the actual necessity of an intermediate host in the spread of the disease and the specificity of the intermediate host. The possibility of hereditary infection among lice must also be considered. Clinically, the exanthema and the characteristically limited course of the disease seem to point to a protozoan cause. Likewise suggestive is the characteristic onset with chills. Finally the Wassermann reaction in typhus fever points to a protozoan-like cause of the disease. Jablons,[13] in 1914, showed that during the florid period of the disease typhus fever may give positive Wassermann complement-deviation in practically 90 per cent. of the cases.

Conclusions. 1. There is no conclusive evidence that the Bacillus typhi-exanthematici of Plotz is the cause of typhus fever.

2. Proteus X19 has more in favor of its being the cause of typhus fever than the Plotz bacillus, but on immunologic and other grounds it appears that both of these bacteria are secondary invaders.

3. There is evidently a state of more or less mixed infection in typhus fever.

4. There may be a state of symbiosis.

5. The Rickettsia prowazeki are probably the cause of typhus fever.

6. The Rickettsia seem to constitute a new group of microorganisms, probably more closely related to the protozoa than to the bacteria.

REFERENCES.

1. Baehr and Plotz: Jour. Infect. Dis., 1917, xx, 201.
2. Brumpt: Bull. Soc. de Path. exot., 1918, No. 11, p. 249.
3. Dienes: Feldarztl. Blätter, No. 11.
4. Dienes: Deutsch. med. Wchnschr., 1917, No. 15, xliii, 461.
5. Doerr and Pick: Wien. klin. Wchnschr., 1918, No. 30, xxxi, 820.
6. Felix: Ibid., 1916, No. 28, xxix, 873.
7. Felix: München. med. Wchnschr., 1917, No. 39, vol. lxiv.
8. Freidberger: Deutsch. med. Wchnschr., 1917, Nos. 42, 43 and 44, vol. xliii.
9. Gavino and Girard: Pub. de inst. Bacteriol. Nacionale de Mexico, 1911.
10. Jungmann: Berl. klin. Wchnschr., 1916, No. 12, liii, 323.
11. Jungmann: Deutsch. med. Wchnschr., 1918, No. 49, xliv, 1346.
12. Jungmann and Kuczynski: Ibid., 1917, No. 12, p. 359.
13. Jablons: Jour. Med. Research, 1914, No. 144, vol. xxv.
14. Landsteiner and Hausmann: Med. Klin., 1918, No. 21, xiv, 515.
15. Munk and Rocha-Lima da: München. med. Wchnschr., 1917, No. 44, lxiv, 1357.
16. Mollers and Wolff: Deutsch. med. Wchnschr., 1919, No. 13, vol. xlv.
17. Nicolle, Blanc and Conseil: Arch. de l'Inst. Pasteur de Tunis, 1914, ix, 84.
18. Nicolle, Conor and Conseil: Ann. de l'Inst. Pasteur, 1911, vol. xxv.
19. Nöller: Berl. klin. Wchnschr., 1916, No. 28, liii, 778.
20. Nöller: Ibid., 1917, liv, 346.
21. Nöller: Arch. f. Schiffs. u. Tropen-Hyg., 1917, xxi, 53.
22. Oettinger: Centralbl. f. Bakteriol., 1918, No. 60, lxxxix, 304.
23. Olitsky, Denzer and Husk: Jour. Infect. Dis., 1916, xix, 811.
24. Plotz: Jour. Am. Med. Assn., 1914, lxii, 1556.
25. Plotz, Olitsky and Baehr: Jour. Infect. Dis., 1915, xvii, 1. (Contains complete Bibliography to 1915.)
26. Plotz, Olitsky and Baehr: Jour. Am. Med. Assn., 1916, lxvii, 1597.
27. Plotz, Olitsky and Baehr: Ibid., 1917, lxviii, 576.
28. Hegler and Prowazek: Berl. klin. Wchnschr., 1913, No. 44, l, 2035.
29. Prowazek: Ztschr. f. Hyg. u. Infectionskrankh., 1916, No. 3, vol. lxxxii.
30. Rabinowitsch: Arch. f. Hyg., 1909, No. 4, clxx, 331.
31. Reder: Das Fleckfieber, published by Franz Deuticke, Leipzig, 1918. (Contains complete Bibliography to 1918.)
32. Ricketts and Wilder: Jour. Am. Med. Assn., 1910, vol. liv.
33. Rocha-Lima da: Arch. f. Schiffs. u. Tropen-Hyg., 1916, No. 20, p. 17.
34. Rocha-Lima da: Berl. klin. Wchnschr., 1916, No. 21, liii, 567.
35. Rocha-Lima da: München. med. Wchnschr., 1916, No. 39, lxiii, 1381.
36. Rocha-Lima da: Ibid., 1917, No. 1, lxiv, p. 33.
37. Rocha-Lima da: Med. Klin., 1917, No. 43, xiii, 1147.
38. Rocha-Lima da: München. med. Wchnschr., 1918, No. 52, lxv, 1454.
39. Sergent, Foley and Vialatte: Compt. rend. Soc. de biol., 1914, p. 101.
40. Sikora: Arch. f. Schiffs. u. Tropen-Hyg., 1916, No. 20.
41. Toepfer: München. med. Wchnschr., 1916, No. 42, xliii, 1495.
42. Toepfer: Deutsch. med. Wchnschr., 1916, No. 41, xlii, 1251.
43. Toepfer: Ibid., xlix, 1517.
44. Toepfer and Schüssler: Ibid., No. 38, p. 1157.
45. Weil: Wien. klin. Wchnschr., 1917, No. 13, xxx, 393.
46. Weil and Felix: Ibid., 1916, Nos. 2 and 31, xxix, 33 and 927.
47. Weil and Felix: Ibid., 1917, No. 48, vol. xxx.
48. Weil and Felix: München. med. Wchnschr., 1918, No. 1, lxv, 17.
49. Widmann: Ibid., 1915, No. 39, lxii, 1336.